Formation and Measurement of Ras Y32 Mutant's Role in GTP Hydrolysis

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Introduction: Members of the Ras protein family are involved in sending signals through the human body to regulate processes including cell growth. Acting as a molecular switch, Ras alternates between its active and inactive states ("on" and "off") depending on its binding to either GTP or GDP. Upon the introduction of a mutation, Ras becomes incapable of performing the GTP hydrolysis reaction at a controlled rate. This results in the protein remaining in the "on" state for prolonged periods of time, disturbing its role in the regulation of cell growth and causing tumors to form. In the genome of cancerous tumors, mutations that permanently activate Ras have been observed and analyzed at three distinct locations, one being Q61. Previous research[1] shows how the Q61 residue contributes to the protein's function by stabilizing the water molecule in the active site, but little research has been done studying the Y32 residue, which has also been implicated in the mechanism. This research investigates the role Y32 plays in activating Ras via introduction of a different amino acid and measurement of the mutated protein's activity in the hydrolysis of GTP.

Materials and Method: Site-directed mutagenesis was used to generate mutations to Ras at position 32. The mutated DNA was transformed into XL10 gold cells and BL21-DE3 cells for gene and protein expression. Cells were grown in TB broth for 24 hours, induced with ITPG, and collected via centrifugation and sonication to break the cells apart. The supernatant was then run through a Ni-NTA purification column that isolates the protein of interest from all other cell particles. A BCA assay was done to measure protein concentration before a kinetics experiment was done over a 6-8 hour time period. A malachite green assay was used to quantify the phosphate byproduct of the hydrolysis reaction.

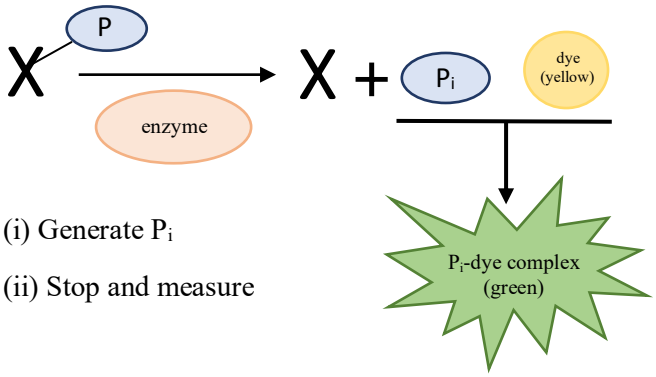

Figure 1. The malachite green assay stops the hydrolysis reaction, phosphate is cleaved from the protein, and the concentration of phosphate in solution is measured via photospectometry.

Results from this assay display the changes in overall phosphate yield and hydrolysis rates over a certain period of time.

Results and Discussion: Demonstrated by the hydrolysis of phosphate from the GTP substrate of the protein, preliminary results show that certain mutants exhibit faster rates of intrinsic hydrolysis while others exhibit slower rates. This indicates that the Y32 residue does plays a role in controlling the rate of intrinsic hydrolysis.

Conclusions: Ras Y32 appears to play a role in the hydrolysis mechanism of GTP because mutations to the protein result in changes to the intrinsic hydrolysis rate. Further investigation of additional mutants, changes in the electric field in the active site, and characterization of potential changes to the protein's structure are necessary to fully understand the role of Y32.

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References: [1]Novelli, E.T.; First, J.T.; Webb, L.J. "Quantitative Measurement of Intrinsic GTP Hydrolysis for Carcinogenic Glutamine 61 Mutants in H-Ras" *Biochemistry* **2018**, *57*, 6356−6366